

# Survival of boll weevil (Coleoptera: Curculionidae) adults after feeding on pollens from various sources

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**Abstract** The survival of overwintering boll weevil, *Anthonomus grandis grandis* (Boheman), adults on non-cotton hosts in the Lower Rio Grande Valley (LRGV) of Texas was examined from 2001 to 2006. The success of the Boll Weevil Eradication Program, which was re-introduced into the LRGV in 2005, depends on controlling overwintering boll weevil populations. Laboratory studies were conducted using boll weevil adults that were captured in pheromone traps from September through March. The number of adults captured per trap declined significantly in the field from fall to the beginning of spring (3.5–7.0-fold). The proportion of trapped males and females did not differ significantly. The mean weight of boll weevil adults captured in September was 13.3 mg, while those of captured adults from November to February were significantly lower and ranged from 6.7 to 7.8 mg. Our results show that boll weevil adults can feed on different plant pollens. The highest longevity occurred when adults were fed almond pollen or mixed pollens (72.6 days and 69.2 days, respectively) and the lowest when they fed on citrus pollen or a non-food source (9.7 days or 7.4 days, respectively). The highest adult survival occurred on almond and mixed pollens [88.0%–97.6% after 1st feeding period (10 days), 78.0%–90.8% after 3rd feeding period (10 days), 55.0%–83.6% after 5th feeding period (10 days), and 15.2%–32.4% after 10th feeding period (10 days)]. The lowest adult survival occurred on citrus pollen [52.0%–56.0% after 1st feeding period (10 days), 13.3% after 3rd and 5th feeding periods (10 days), and 0 after 6th feeding period (10 days)]. Pollen feeding is not a behavior restricted to adult boll weevils of a specific sex or physiological state. Understanding how boll weevil adults survive in the absence of cotton is important to ensure ultimate success of eradicating this pest in the subtropics.

**Key words** boll weevil, *Anthonomus grandis grandis*, Curculionidae, subtropics, pollen  
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## Introduction

The boll weevil, *Anthonomus grandis grandis* (Boheman), feeds and oviposits on cotton (*Gossypium hirsutum* L.).

Both male and female adults cause damage by feeding, while only females damage fruiting structures during oviposition. The larvae feed within the fruit and cause extensive damage. Bracts on damaged flower buds (i.e. squares) open pre-maturely, and these damaged squares are usually aborted by the plant (Leigh *et al.*, 1996).

Boll weevil adults overwinter in the southern U.S., utilizing cotton stalks as the primary diapausing habitat. In the Lower Rio Grande Valley (LRGV) of Texas, the Boll Weevil Eradication Program has established a deadline

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when commercial cotton must be harvested and remaining stalks destroyed. This greatly reduces the ability of adult populations to overwinter and then reproduce to infest newly planted cotton fields the following spring (Walker & Smith, 1996).

In the LRGV of Texas, diapausing adults do not reproduce, and their metabolic activity is suppressed (Wolfenbarger *et al.*, 1976; Graham *et al.*, 1978, 1979; Guerra *et al.*, 1982; Summy *et al.*, 1993). Diapause functions to enhance the survival of boll weevils during periods of food shortage (Guerra *et al.*, 1984; Summy *et al.*, 1988). Leggett and Fye (1969) reported that adults increased in remaining cotton stalks and survived through the winter until newly planted cotton was available in the spring. Large numbers of adult boll weevils captured in pheromone traps in the winter may indicate that overwintering populations may be physiologically active and seeking hosts (Bariola *et al.*, 1984); however, researchers are still uncertain regarding the overwintering habitat. Alternative foraging resources play a significant role in adult boll weevil survival, especially in the absence of cotton (Jones *et al.*, 1993; Hardee *et al.*, 1999). Boll weevil adults primarily feed on pollen from the Malvaceae family (Cate & Skinner, 1978); however, they also feed on pollen from plants in other taxa (Cross *et al.*, 1975; Burke *et al.*, 1986; Benedict *et al.*, 1991; Jones & Coppedge, 1999). These plants may provide energy and nutrients, in the form of pollen, that increases adult survival in the absence of cultivated cotton; however, it is not known if boll weevil adults will feed on pollen from commonly grown crops in subtropical LRGV. In addition, the effects of alternative foraging resources on overwintering adults have not been thoroughly investigated.

The objective of this study was to determine adult survivability on plants that may serve as hosts in the subtropics when cotton is unavailable, especially during the winter months.

## Materials and methods

### *Capture of overwintering boll weevil adults*

This study was conducted during September to March, 2001–2006 at two experimental plots at the USDA-ARS-Subtropical Agricultural Research Center in Weslaco, Texas (North and South Farms). A privately owned cotton field (Brownsville, Texas) and two wildlife refuges (Santa Ana and Donna) adjacent to cotton fields were used as additional sites. All experimental sites had been planted with cotton and had a history of boll weevil infestations. Boll weevil adults used in the study were collected from pheromone traps. Traps were placed around the perimeter of

each experimental site (10 traps at South Farm, 15 traps at North Farm, 20 traps at Brownsville, 10 traps at Santa Ana and 10 traps at Donna) approximately 1.2 m above the ground. The traps were positioned 50 m apart. Each trap contained a grandlure dispenser that was replaced every 10 day. Both traps and grandlure dispensers were purchased commercially (Plato Industries, Houston, TX, USA or Great Lakes IPM Co., Vestaburg, MI, USA). Trapping occurred from September through March, and traps were inspected once every 10 days. Captured boll weevil adults were transported to the laboratory. Number of boll weevil adults was recorded, and the mean number of adults per trap was calculated (number of captured adults was divided by the number of traps). Adults were sexed (Sappington & Spurgeon, 2000) and weighed using an analytical balance (Mettler Instrument Corp., Hightstown, NJ, U.S.). In addition, three fat body categories (i.e. fat, intermediate, and lean) were recorded as described by Spurgeon *et al.* (2003).

### *Boll weevil adult feeding on selected plant pollens*

Five pollen types were used as a food source: almond, *Prunus dulcis* (Mill.) Webb; citrus, *Citrus* spp.; corn, *Zea mays* L.; melon, *Cucurbita melo* L. subsp. *melo*; and a mixed pollen source. These sources, except almond, all occur in the LRGV of Texas. Almond pollen was used as a positive control because it makes copious amounts of pollen that was easy to obtain without interfering with honeybee activity. Almond pollen was collected in the vicinity of Bakersfield, CA, USA. A pollen analysis of the mixed source was determined by counting and identifying 1 000 grains that occurred in the mixed pollen types. The main pollen types included low spine Asteraceae (25%) including *Iva* sp. and *Parthenium* sp.; Fabaceae (15%) containing *Leucaena* sp.; Cheno-Ams (10%) (morphological type representative of Chenopodiaceae and Amaranthaceae); Poaceae (10%) including corn; *Morus* sp. (5%); and *Helianthus annuus* C. Linnaeus (3%). All pollens were collected by honeybees in 2004–2006 from a large area of cultivated crops (~100 acres or more of each) and widely distributed weeds (mixed pollen). Modified Ontario Agricultural College Honeybee Pollen Traps (purchased either from Mann Lake Limited, Hackensack, MN or Dadant and Sons, Incorporated of Hamilton, IL, U.S.) were used to trap the pollen pellets from foraging honeybees. Traps were installed in honeybee colonies located in standard Langstroth hives (Schmidt & Buchmann, 1992). This is a bottom pollen trap. In the process, one or more corbicular pollen loads is dislodged and falls into a collection tray located at the bottom of the trap. Pollen collection trays were emptied every 24 or 48 h (age of

pollen used in the assays). Pollens were kept frozen ( $-20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) until used (about 1 year or less without losing their nutritional values, Haydak, 1963; Dietz & Stevenson, 1980; Shivanna & Johri, 1985). For the pollen feeding experiments, we used 24-h-old trapped boll weevil adults in pheromone traps that were cleaned 24 h before collection. Any trap-collected adults were starved for 72 h, but were provided with distilled water (cotton wick saturated with water), to clear the digestive tract of food consumed before starting the laboratory assays (Cate & Skinner, 1978). Five males or females were held in 15-cm diameter Petri dishes [each dish was ventilated by a 4-cm diameter circular screened hole (organdy cloth) in the lid] in an environmental chamber (Percival Scientific Inc., Boone, IA, US 50036) at  $27 \pm 1^{\circ}\text{C}$ , 65% RH, and a photoperiod 12:12 (L:D) h. Temperature and humidity were monitored using a Fisher-brand Traceable Relative Humidity Meter with temperature readout (Fisher Cat. No.11-661-12, Control Company, Friendswood, TX, U.S.). Pollen (1 mg/adult) was mixed in a single drop of distilled water and provided daily for the adults. Adults were provided pollen until death to estimate longevity and survival (2004–2006). Adults were considered to be dead if they did not respond to probing with a dissecting needle. Feeding assays with melon (25 replications/sex), corn (15 replications/sex), and citrus (15 replications/sex) were conducted only once, using adults captured from late October to early November. The mixed pollens (50 replications/sex) and almond pollen (20–25 replications/sex) were used three times for feeding overwintering adults captured in late October to early November, late November to early December, and late December to early January. Non-fed boll weevils (50 replications/sex), before they died, were used as a control.

For estimating the number of adults with pollen and number of pollen grains in their gut (2005–2006), we fed with pollens described above 15–25 boll weevils each sex for 7–10 days. Later, those adults were removed and dissected. The gut was dissected from male and female adults, and contents were acetolyzed and the residue was examined for the presence of pollen using the methods described by Erdtman (1960) and Jones and Coppedge (1999).

#### Statistical analyses

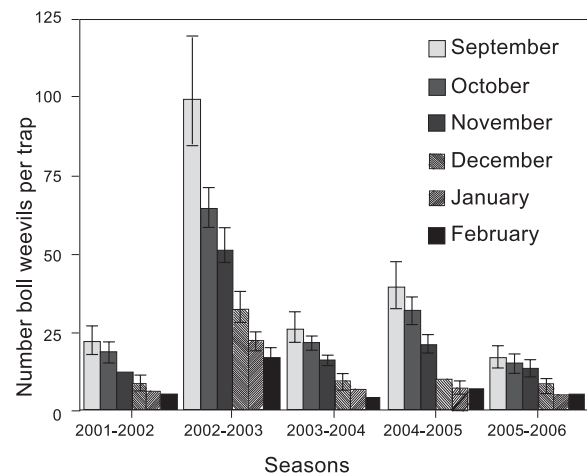
For each sex, longevity of overwintering adults feeding on different pollens, the number of captured overwintering adults trapped, and the mean adult weight were subject to a one-way analyses of variance (ANOVA) using PROC GLM (SAS Institute, 1999). Means were separated using a Tukey-Kramer test (TUKEY option of the LSMEANS statement) (SAS Institute, 1999). The influence of pollen source on subsequent

adult survival was analyzed separately by sex. The body fat of females and males were compared among months using a log-likelihood *G*-test (Zar, 1999). Pollen grains recovered from overwintering boll weevils maintained on different pollen food sources were analyzed descriptively.

## Results

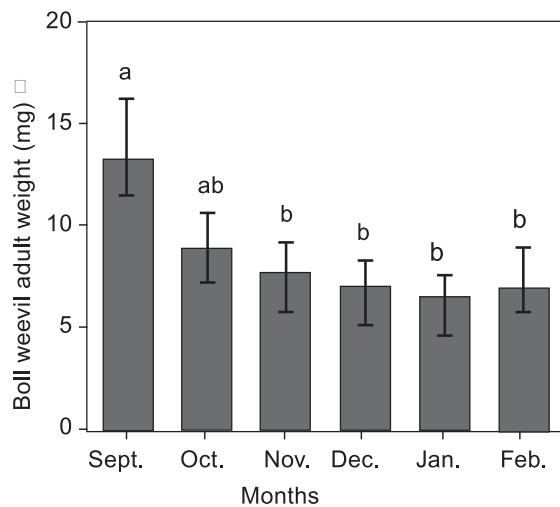
### Capture of overwintering boll weevil adults

As expected, the number of captured adults per trap decreased from September through February (Fig. 1). The percentage of adult reduction, from the post-harvest period to the beginning of spring, was 76.3% (2001–2002) ( $P = 0.001$ ) and 68.7% (2005–2006) ( $P = 0.009$ ), likely related to boll weevil adult mortality. The same trend was observed in 2002–2003 when reduction of captured adults was 81.0% ( $P = 0.001$ ); in 2003–2004 when reduction was 85.7% ( $P = 0.001$ ); and in 2004–2005 when it was 82.4% ( $P = 0.001$ ) (Fig. 1). There was no significant difference in males and females captured. The percentage of trapped females for 2001–2002 was 43.3%–53.4%, while males 46.6%–56.7% ( $F = 0.9$ ;  $\text{df} = 5, 90$ ;  $P > 0.05$ ), and for 2005–2006 was 43.9%–50.0% and 50.0%–56.1%, respectively ( $F = 0.7$ ;  $\text{df} = 5, 90$ ;  $P > 0.05$ ).



**Fig. 1** Number of captured boll weevil adults per pheromone trap in different overwintering seasons.

The weight of captured boll weevil adults decreased during the winter months. The mean weight (mg) for adults captured in September was 1.5-fold higher than adults captured in October, and significantly (1.8-fold) higher than mean weight of boll weevil adults captured for the next 4 months (November through February) ( $F = 58.8$ ;  $\text{df} = 5, 732$ ;  $P = 0.001$ ) (Fig. 2).



**Fig. 2** Mean weight (mg) of captured overwintering boll weevil adults.

#### Boll weevil adult feeding on selected plant pollens

Overall, a greater percentage average for three months of adults with pollen in their gut was observed when fed almond pollen (92.0% of males and 100% of females) or mixed pollen, 93.3% and 95.5%, respectively (Table 1). Also, adults that fed on almond pollen contained the highest number of pollen grains per boll weevil (15.2 grains/male and 19.1 grains/female) followed by those that fed on mixed pollen (7.7 grains and 8.2 grains, respectively) (Table 1). Citrus pollen (3.7 grains/female and 6.5 grains/male) was present in the gut of only 31% of females and 30% of males. The lowest percentage of adults with pollen in their gut was observed for those that fed on melon pollen (24% in males and 18% in females). Adults that fed on melon pollen had the lowest number of pollen grains present in their gut (1.3 pollen grains/male and 2.1 pollen grains/female).

**Table 1** Pollen grains recovered from overwintering boll weevils maintained on different pollen food sources<sup>†</sup>.

Food sources	Month of feeding	Number examined		Percentage of adults with pollen in gut		No. pollen grains per boll weevil	
		Male	Female	Male	Female	Male	Female
Mixed pollen	October	25	25	84.0	87.5	3.1	3.6
	November	25	25	96.0	92.0	7.2	6.8
	December	25	25	100.0	100.0	12.7	14.3
Almond	October	25	25	100.0	100.0	8.6	12.7
	November	25	25	96.0	100.0	21.3	24.9
	December	25	25	80.0	100.0	15.8	19.7
Citrus	December–January	20	20	30.0	31.0	6.5	3.7
Corn	October–November	15	15	35.0	37.0	8.2	10.9
Melon	October–November	20	20	24.0	18.0	1.3	2.1

<sup>†</sup>Traditionally, pollen studies simply use descriptive statistics. Thus, the data presented in this table were descriptively analyzed.

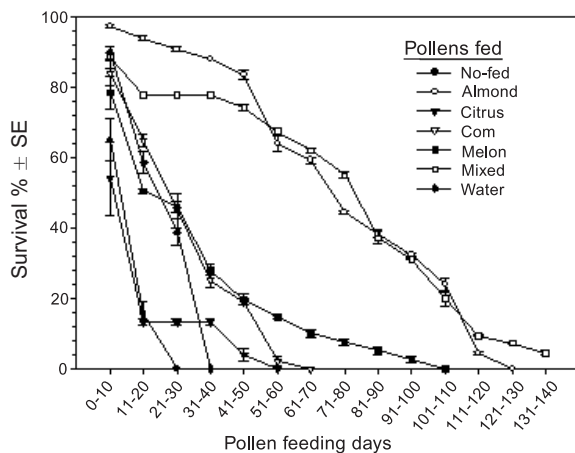
**Table 2.** Effects of different pollens on longevity of overwintering boll weevils.

Pollen sources	Captured time periods	Longevity, mean $\pm$ SE <sup>†</sup> days			
		Males		Females	
		Days $\pm$ SE	Range	Days $\pm$ SE	Range
Almond	October–November	72.6 $\pm$ 6.5 aA	4–121	57.8 $\pm$ 7.0 aA	1–129
Almond	November–December	31.8 $\pm$ 7.5 bA	2–96	27.6 $\pm$ 6.2 aA	2–85
Almond	December–January	30.6 $\pm$ 5.6 bA	2–67	12.4 $\pm$ 1.8 bcA	1–27
Citrus	October–November	9.7 $\pm$ 3.4 cA	2–44	9.9 $\pm$ 3.5 cA	3–44
Corn	October–November	24.2 $\pm$ 5.2 bA	3–52	26.4 $\pm$ 4.4 bA	4–52
Melon	October–November	35.6 $\pm$ 6.8 bA	3–110	29.0 $\pm$ 5.4 bA	3–77
Melon	November–December	25.6 $\pm$ 5.7 bA	2–95	26.4 $\pm$ 6.6 bA	1–89
Melon	December–January	29.4 $\pm$ 5.5 bA	2–76	26.2 $\pm$ 5.3 bA	1–94
Mixed	October–November	69.2 $\pm$ 5.8 aA	3–145	54.6 $\pm$ 5.3 aA	3–140
Water		17.3 $\pm$ 1.6 bcA	1–31	18.0 $\pm$ 1.6 bA	2–37
Non-fed		7.4 $\pm$ 0.7 cA	1–21	4.7 $\pm$ 0.4 cA	1–16

<sup>†</sup>Means within a column followed by the same lower case letter are not significantly different (Tukey-Kramer test,  $P > 0.05$ ). Pairs of means within a row followed by the same upper case letter are not significantly different (Tukey-Kramer test,  $P > 0.05$ ).

Adult longevity of males was higher after feeding on almond pollen ( $72.6 \pm 6.5$  days, with a maximum of 121 days and minimum of 34 days) or the mixed pollen ( $69.2 \pm 5.8$  days, with a maximum of 145 days and minimum of 3 days) from October through November (Table 2). Adult longevity of males was the shortest after feeding on citrus pollen ( $9.7 \pm 3.4$  days, with a maximum of 44 days and minimum of 2 days) and no food ( $7.4 \pm 0.7$  days, with a maximum of 21 days and minimum of 1 day) ( $F_{\text{males}} = 22.1$ ;  $df = 10, 292$ ;  $P = 0.001$ ). The same trend was observed for females ( $F_{\text{females}} = 14.8$ ;  $df = 10, 272$ ;  $P = 0.001$ ). Pollen source and time of feeding (months) (in the laboratory for boll weevils collected from traps in the field and when fed with pollen at 24–48 h) had no significant effect on adult longevity, except after feeding on almond pollen. Male and female longevity was not significantly different after feeding on the same pollen source ( $t$ -tests ranged from 0.1 to 0.968).

Pollen source influenced the survival of adult boll weevils in the absence of cotton. Adults presented with pollen had a significantly greater survival rate than individuals



**Fig. 3** Survivorship profiles of overwintering boll weevil adults fed on different pollens for every 10-day period.

with no food or only water (Fig. 3). Adult survival was greater after feeding on almond pollen (97.6% males and 88.0% females after 1st 10 days feeding, 94.0% and 83.2%, respectively, after 2nd 10 days feeding, 90.8% and 80.0%, respectively, after 3rd 10 days feeding, 83.6% and 70.8%, respectively, after 5th 10 days feeding, 44.4% and 32.0%, respectively, after 8th 10 days feeding, and 32.4% and 16.0%, respectively, after 10th 10 days feeding) or fed with mixed pollen (88.6% males and 95.4% females after 1st 10 days feeding, 78.0% and 82.2%, respectively, after 2nd 10 days, 78.0% and 78.0%, respectively, after 3rd 10 days, 74.2% and 52.4%, respectively, after 5th 10 days, 55.0% and 27.6%, respectively, after 8th 10 days, and 31.2% and 15.2% males and females after 10th 10 days period). The lowest adult survival occurred after feeding on citrus pollen (54.0% males and 56.0% females after 1st 10 days feeding, 13.3% and 13.3%, respectively, after 2nd, 3rd, 4th, and 5th 10 days feeding, and 0% after 6th 10 days feeding) (Fig. 3) ( $F_{\text{females}} = 10.0$ ;  $df = 6, 528$ ;  $P = 0.001$  and  $F_{\text{males}} = 18.8$ ;  $df = 6, 523$ ;  $P = 0.001$ ). Male and female survival was not significantly different after feeding on the same pollen source ( $t$ -tests ranged from 0.226 to 0.984), except feeding on almond ( $t$ -test was 0.001) and mixed pollen ( $t$ -test was 0.017), where survival of males were significantly higher. Fat body content of females feeding on pollen declined significantly from the fall to spring (from 21.7% scored fat, 28.6% intermediate, and 49.7% lean in September greater than 90.9% rated as lean during December through March (Table 3). Most males captured from September to March were rated as lean (82.5%–100.0%).

## Discussion

Our data showed a reduction in the numbers of boll weevil adults captured in pheromone traps over the winter. Graham *et al.* (1979) and Guerra *et al.* (1982) also observed

**Table 3.** Percent fat body distribution of overwintering boll weevil adults (females/males).

Month	Distribution of fat body rating <sup>†</sup> , %			G-test fat body value <sup>‡</sup>	Mean fat body rating
	Fat	Intermediate	Lean		
September	21.7 / 0.0	28.6 / 5.0	49.7 / 95.0	62.8	1.7 ± 0.4 / 1.1 ± 0.05 a
October	8.2 / 0	19.7 / 17.5	72.1 / 82.5	12.2	1.4 ± 0.2 / 1.2 ± 0.02 a
November	0 / 0	22.6 / 0	77.4 / 100	34.2	1.2 ± 0.03 / 1.0 a
December	0 / 0	9.1 / 0	90.9 / 100	13.0	1.1 ± 0.01 / 1.0 a
January	0 / 0	0 / 0	100 / 100	0.0	1.0 / 1.0 a
February	0 / 0	0 / 0	100 / 100	0.0	1.0 / 1.0 a
March	0 / 0	1.3 / 2.5	98.7 / 97.5	0.4	1.01 ± 0.01 / 1.0 ± 0.02 a

<sup>†</sup>Fat = 3.0, Intermediate = 2.0, Lean = 1.0.

<sup>‡</sup>Significant difference between females and males fat body ( $\chi^2 = 5.991$ )  $P_{\text{females}} = 0.9$ ;  $P_{\text{males}} = 1.0$ .



that the number of adult boll weevils captured in traps in the LRGV peaked in September and then declined in spring. Guerra *et al.* (1982) reported that males were always more abundant than females while a slight variation in the sex ratio of trapped adults in Arizona (1:1 to 1.2:0.8) was observed by Sivasupramaniam *et al.* (1995). However, we observed no significant differences between captured males and females in the LRGV. Furthermore, trapping data should not be directly related to changes in boll weevil populations during the overwintering period, because the proportion of the population sampled is unknown. However, our data indicated a clear tendency of a reduction in adult numbers trapped during the winter in the LRGV, which is likely associated with adult mortality. Summy *et al.* (1993) and Bodden (1997) also associated the reduction in adults trapped during the overwintering period with mortality, and attributed the absence of cotton fruit as the main factor. Researchers demonstrated the relative cold tolerance of boll weevil adults (Slosser *et al.*, 1994; Suh *et al.*, 2002), and more than 90% of non-diapausing boll weevils tolerated freezing temperatures of 0.0°C and -2.5°C for up to 8 h (Slosser *et al.*, 1994). However, Fye *et al.* (1970), Guerra *et al.* (1984), and Summy *et al.* (1988) noted that adults deprived of cotton as a food source are unable to overwinter successfully in subtropical and tropical regions. In addition, in the absence of cotton, adults typically weighed less. Greenberg *et al.* (2005) demonstrated significant decreases for the smallest of adults in terms of longevity, oviposition rates, and the survival of progeny. Reduction in weight of overwintering boll weevil adults can be used for prediction of significant decreases in survival, development, and reproduction of their progeny in spring time on cotton.

One important component that needs to be evaluated is the affect of alternative food sources on adult longevity. There are few studies that investigate the influence on longevity of overwintering adults fed on plants that serve as food sources in the absence of cotton that provide alternative feeding sites (Chandler & Wright, 1991).

Fenton and Dunnam (1929) showed that longevity of overwintering adults after emergence is quite short if alternative food sources are not available. Fye *et al.* (1959) determined average longevity of overwintering adults that fed on fruiting cotton to be 21.9 days, whereas Fenton and Dunnam (1929) determined adult longevity to be only 8.1 days. Haynes and Smith (1992) found some laboratory adults survived for 2–3 weeks after feeding on the following diets: 1% pollen and 1% sugar, primrose sp. (*Primula*), or sow thistle (*Sonchus*). Some adults survived for  $\geq 5$  weeks on a diet of unadulterated honey, as well as bee-collected pollen plus water.

Many subtropical and tropical beetles are pollen feeders and survive host-free periods as active, non-reproductive

adults that feed on a variety of plant pollens and nectar (Janzen, 1980). This is a viable survival mechanism in the subtropics and tropics because flowering plants are typical throughout the year, even during markedly dry seasons (Frankie, 1973). Pollen from different plant species varies in nutritional value to pollen-feeding insects (Barbier, 1970; McCaughy *et al.*, 1980).

The extensive variety of pollen present in the gut of boll weevil adults suggests that the degree of selectivity in the choice of feeding hosts is low. Pollen from the Fabaceae and Asteraceae plant families is more common and more highly aggregated than pollen from the Malvaceae plant family during non-productive periods in the boll weevil life cycle in northeastern Mexico (Jones *et al.*, 1993). According to Benedict *et al.* (1991), in the LRGV, the most common types of pollen consumed by boll weevil adults are from the Poaceae, Brassicaceae, and Asteraceae plant families. Mitchell and Taft (1966) indicated that boll weevil adults needed very little fat reserve to survive the winter. Our data have confirmed other previous studies that demonstrated pollen feeding is apparently not a behavior restricted to boll weevil adults of a specific sex or physiological state (Jones, 1997).

Adult boll weevils are polyphagous pollen feeders and actively feed on pollen from a diverse variety of plant species. Pollen consumption may be one of the principle evolutionary survival mechanisms of adults in fall and winter in absence of cotton in the LRGV. The reduction of overwintering boll weevil populations is a key management strategy in control of the pest and is a principal component in area-wide eradication programs. Understanding how boll weevil adults survive on plants other than cotton is important in how to eradicate this pest, especially in the subtropics.

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